FREQUENCY OF THE CDH1 GENE MUTATIONS IN THE DIGESTIVE CANCER

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Abstract: *Genetic alterations associated with the development of digestive cancer are often observed. The most frequent mutations are small deletions or insertions. The aim of this study was to detect CDH1gene mutations encompassing 16 exons by MLPA technique (Multiplex Ligation – dependent Probe Amplification) in a group of 12 patients with digestive cancer. Genomic DNA isolated from blood, tumour tissue and healthy tissue (as a control for MLPA) was amplified by PCR and than analyzed by capillary electrophoresis on ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems). In the case of MLPA analysis 58.33% of patients had alteration such as deletion or insertion. These results will be helpful to understand the mutation spectrum of the frequent CDH1 gene involved in digestive cancer pathology. Our study continues with larger samples required to establish a genetic profile for both high-risk and Romanian cancer patients with sporadic digestive cancer.*

Cuvinte cheie: *cancer digestiv, gena CDH1, MLPA*

Rezumat: *Alterările genetice asociate cu dezvoltarea cancerului digestiv sunt adesea observate. Cele mai frecvente mutații sunt deleţiile mici □i inserţiile. Scopul acestui studiu a fost de a detecta mutaţiile genei CDH1 care cuprinde un număr de 16 exoni, prin tehnica MLPA (Multiplex Ligation – dependent Probe Amplification) la un grup de 12 pacien□i cu cancer digestiv. ADN genomic a fost izolat din sânge, ţesut tumoral şi ţesut sănătos (control pentru MLPA) şi amplificat prin PCR, iar apoi analizat prin electroforeză capilară pe aparatul ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems). În cazul analizei MLPA, 58.33% dintre pacienţi au avut alterări de tipul deleţiei sau inserţiei. Aceste rezultate vor fi folositoare pentru înţelegerea spectrului mutaţional al frecvenţei genei CDH1 implicată în patologia cancerului digestiv. Studiul nostru va continua pe un lot mai mare de probe necesare pentru a stabili profilul genetic atât pentru indivizii cu risc crescut, cât şi pentru pacienţii români cu cancer digestiv sporadic.*

INTRODUCTION

Gastric cancer is the second leading cause of cancer mortality after lung cancer and is responsible for more than 10% of deaths caused by cancer, but its incidence worldwide is continuously decreasing (due to the identification of some risk factors such as *Helicobacter pylori* infection, environmental factors and food).

Every year, approximately 87000 new cases are diagnosed with gastric cancer. The national prevalence is 2.9 /100000 inhabitants in the adult population, who addressed at the digestive endoscopy services in the country. The incidence is higher in Transylvania (6.6 /100000) and lower in Muntenia (1.0 /100000) according to "*The National multicenter study on prevalence of gastric cancer from Romania*" (2003). Colorectal cancer is the third most common cancer in men (663000 cases, 10.0% of the total) and in women (571 000 cases, 9.4% of the total) worldwide.

Most cases are detected in an advanced stage of disease, because early diagnosis is possible in a higher percentage only in countries using cancer screening.

Numerous studies have identified a genetic predisposition, immunohistochemistry highlighting relevant genetic markers in cancer, some of them correlating with tumor phenotype, and others offering clues on the prognosis or response to chemotherapy. Several genes have been analyzed and a number of changes were identified at theirs level, including deletion, suppression, amplification, overexpression, microsatellite instability, DNA aneuploidy.

CDH1 is one of the genes which mutation is found in up to 50% of cases with hereditary diffuse gastric cancer. This mutation has an autosomal dominant pattern of inheritance, the risk of developing advanced forms of cancer being 40-67% for men and 60-83% for women with a mean age of occurrence at the age of 38 years.

It has been demonstrated that loss of E-cadherin function is implicated in the pathogenesis of sporadic colorectal cancer and both sporadic and hereditary forms of difuse gastric tumorigenesis (Machado et al., 1999; Becker et al., 1994).

CDH1 or E-cadherin is a classical cadherin from the cadherin superfamily. The encoded protein is a calcium dependent cell-cell adhesion glycoprotein, and plays a major role in the process of intercellular adhesion between epithelial cells (Takeichi, 1991).

Reduced expression of E-cadherin is regarded as one of the main molecular events involved in dysfunction of the cell-cell adhesion system, triggering cancer invasion and metastasis. Loss of function is thought to contribute to progression in cancer by increasing proliferation, invasion, and/or metastasis.

*****The first two authors have an equal contribution at the realization of this study/article

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THE AIM OF THE STUDY

Our goal was to determine the incidence of copy number variants in cancer predisposition CDH1 gene from unrelated Romanian patients with gastric and colorectal.

MATERIAL AND METHOD

Patient information. The study involved specimens of gastric and colorectal lesions of patients who underwent surgery at the Emergency University Hospital of Bucharest, and the Emergency Clinical Hospital of Bucharest. Samples were collected in the Biochemistry and Molecular Biology Center and staged according to TNM system classification by the pathologists in the Pathological Department of Hospitals involved in the study. Our studied group comprised specimens from 12 patients with gastric (3) and colorectal (9) cancer. Patients gave informed consent according to institutional guidelines prior to surgery.

DNA isolation. DNA samples were extracted from tumour tissue and a safe margin of healthy tissue around it, and blood originating from the same patient (*WizardR Genomic-DNA Purification Kit - Promega*).

MLPA analysis. The MLPA test for CDH1 ensures a sensitive and a high-thtoughput screening test for genomic rearrangement. Screening for all exons of CDH1 was performed according to the instruction of the manufacuturer using the MLPA kit (SALSA P083-B1). MLPA reagents were purchased from MRC-Holland, Amsterdam, and NL. All reactions were carried out on a standard Termocyler (Gene Amp PCR 2700, Applied Biosystem). The DNA-samples were diluted in two hundred nanograms DNA with TE into a final volume of 5µl and denatured at 98°C for 5 min before the addition of 3 µl SALSA Probe mix and MLPA buffer. The reaction mixture was denatured for 1 min at 95°C and incubated for 16 h at 60°C to ensure specific hybridisation of the oligonucleotide probes with their target sequences. This was followed by ligation of the annealed probe with a buffer/Ligase-65 mixture (32 µl) and incubated at 54°C for 15 minutes. The ligation was then continued by heating to 98°C for 5 min. A fluorescent multiplex PCR amplification using a single universal primer pair suitable for all 32 probes was carried out using standard conditions. PCR product (0.75µl) was mixed with 0.6µl of GeneScan-500 ROX TM used as a size standard marker and 14.25 µl of deionised formamide. The analysis time was 35 min using a 47 cm capillary at 60°C. We included three control samples from clinicaly healthy individuals in each MPLA test. Multiplex ligation-dependent probe amplification PCR products were separated on a fluorescent capillary sequencer (ABI PRISM^{TM} 310 Genetic Analyzer, Applied Biosystems). Analysis of the fragments was performed using the GeneMapper ID v3.1 software.

RESULTS

For each exon of the CDH1 gene (16 in totaly) we performed mutation analysis. The P083-B1 probemix used contains probes for each CDH1 exon. CDH1 gene spans ~98.2 kb of genomic DNA and is located on chromosome 16q22.1, ~67.4 Mb from the p-telomere.

Using MLPA we detected 7 patients with aberrant exon copy numbers (Table 1). All samples (DNA from blood, healthy tissue and tumors) from unrelated patients were direcly analysed and no positive cases we found in the blood DNA samples which shows us the sporadic character of gastric and colorectal cancer.

The most affected exons with insertions were the following: 3 (33.3%) and 9 (25%), 4 (8.33%), 8 (8.33%). Three deletions (HO loss) were identified: a deletion of exon 1A, other of exon 2 in patient 5, and one in patient 3 (exon 9). Interestingly, in the female patient 3 we observed a gain in combination with LOH and HO loss.

PCR amplification was sucesely accomplished for all 32 probes: 16 exons (with the remark that exon 1 was divided in two-01A, 01B) and 15 reference probes (internal control) that detect several different autosomal chromosomal locations (Figure 1). This allowed us to further process each tumoral sample in the Coffalyser software (Figure 2 and 3).

LOH (loss of heterozigosity) a diferent type of deletion was observed in patient 5, at the level of exons 6-12, 16, and patient 3 (1B, 2, 7, 12). Furthermore, in patients investigated in this study where gain mutations were found we did not identified any other type of genomic rearrangement.

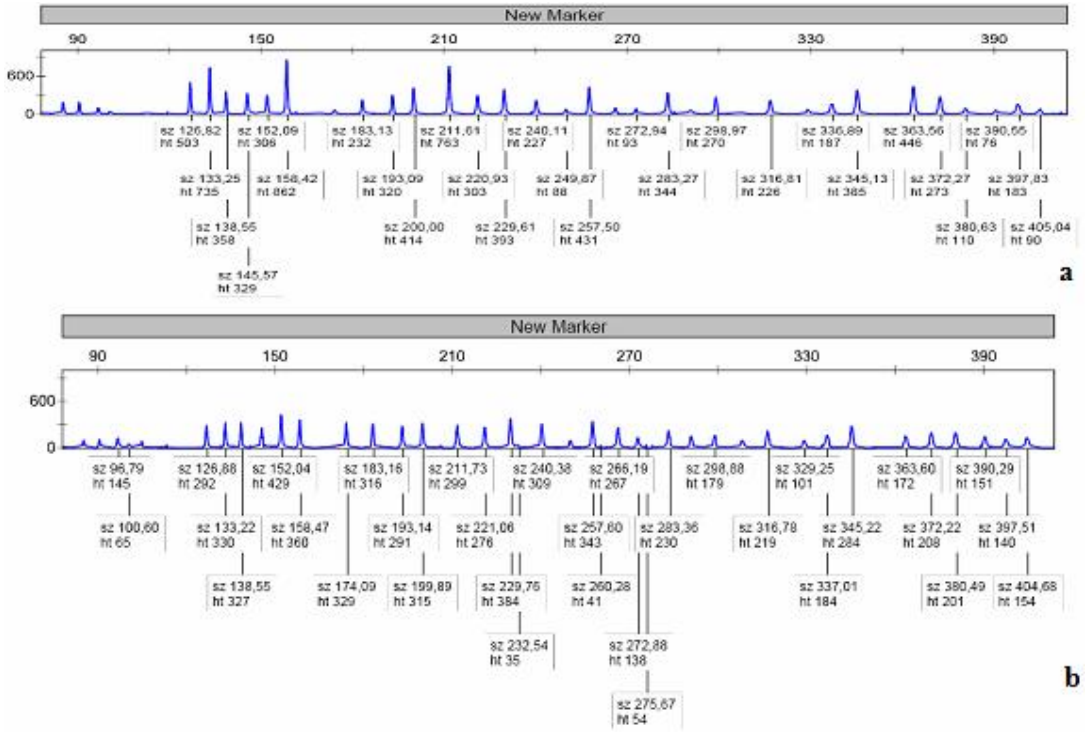
Table no. 1. Evaluation of the genomic rearrangements in samples from genomic DNA isolated from tumors

EXONS	Patient	1 GC	2 GC	3 GC	4 CR	5 CCR	6 CCR	7 CR	8 CCR	9 CCR	10 CCR	11 CCR	12 CCR
	Gender	M	M	F	F	F	M	M	M	F	M	M	M
	Age	70	60	55	80	50	71	71	68	42	81	62	57
	TNM	T4N2M0	T3NXMX	T3N0MX	T3N1M0	T4N1M1	T4N2MX	T3N1M(	T3N0M1	T3N0MX	T3N0MX	T4N0M0	T3N0M0
Exon 01A													
Exon 01B													
Exon 02													
Exon 03													
Exon 04													
Exon 05													
Exon 06													
Exon 07													
Exon 08													
Exon 09													
Exon 10													
Exon 11													
Exon 12													
Exon 13													
Exon 14													
Exon 15													
Exon 16													

GC- gastric cancer; CCR- colorectal cancer; F-female; M-male

ambiguous | gain | LOH | HO loss

Figure no. 1. Chromatogram profile of a tumor sample DNA (a), compared with normal control DNA (b)



DISCUTIONS

Here we performed MLPA to detect copy number changes of CDH1 human gene that often have an increased copy number in one or more types of tumors including gastric and colorectal cancer. According to Frances et.al germline CDH1 mutations predispose to early onset colorectal cancer (Frances, 1999). Other studies investigated CDH1 as a cause of inherited susceptibility to both gastric and colorectal cancers. In difuse gastric carcinoma, despite common CDH1 gene mutations, tumors show absence of CDH1 loss of heterozigosity (LOH) in most cases (Machado 2001). Individuals with abnormal E-cadherin face a lifetime risk of 75-80% of developing diffuse gastric cancer and women have an additional 39% risk of developing lobular breast cancer (Guilford, 1998). In approximately 70-90% of the patients, gastric cancer is sporadic and 10-30% is familial. Among familial gastric cancer cases, only approximately 1-3% of all cases are carriers of mutations in *CDH1*. Mutations in *CDH1* gene have been reported in families with a hereditary predisposition to gastric cancer. Criteria for hereditary diffuse gastric cancer is based on histologically confirmed DGC in three first degree family members at any age, or two or more gastric cancers in first degree relatives with at least one confirmed DGC diagnosed before age 50. Some studies reported that 4% of these mutation positive families exhibited large germline deletions of CDH1 that were not detectable by conventional DNA sequencing, and the recommendation is to use other methods such as multiplex ligation dependent probe amplification -MLPA or alternative methods (array comparative genomic hybridisation -CGH).

Frequent somatic mutations (50%) have been identified only in sporadic diffuse gastric cancer (DGC), and Lobular Breast Cancer (LBC). In these types of carcinoma there is a major difference between the mutation types identified. In diffuse gastric carcinomas, the predominant mutations are exonskippings causing in-frame deletions. By contrast, most mutations identified in the lobular breast cancer are premature stop codons. In the case of the diffuse gastric carcinomas, a mutation cluster region is suggested as more than 60% of mutations cause exon skipping of exon 8 and 9, results that are in accordance with our findings. Our study suggests that the confirmation of MLPA results reveals novel interesting rearrangements in other CDH1 gene exons which may be infrequent in our population.

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REFERENCES

1. Machado JC, Oliveira C, Carvalho R, Soares P, Berx G, Caldas C, Seruca R, Carneiro F, and Sobrinho -Simoes M. E-cadherin gene (CDH1) promoter methylation as the second hit in sporadic difuse gastric carcinoma. Oncogene 20: 1525 - 1528, 2001
2. Machado JC, Soares P, Carneiro F, Rocha A, Beck S, Blin N, Berx G, Sobrinho-Simões M. E-cadherin gene mutations provide a genetic basis for the phenotypic divergence of mixed gastric carcinomas.Lab Invest. 79(4): 459-65, 1999
3. Takeichi M. Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251: 1451-1455, 1991
4. BeckerKF, AtkinsonMJ, ReichU, Becker I, NekardaH, SiewertJR, Hofler H. E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. CancerRes54: 3845-3852, 1994
5. Frances M. Richards, Shane A. McKee, M. Helen Rajpar, Trevor R. P. Cole, D. Gareth R. Evans, Janusz A. Jankowski, Carole McKeown, D. Scott A. Sanders and Eamonn R. Maher. Germline E-cadherin gene (CDH1) mutations predispose to familial gastric cancer and colorectal cancer.Human Molecular Genetics, Vol. 8, No. 4 607–610, 1999
6. Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, Harawira P, Taite H, Scoular R, Miller A, Reeve AE. E-cadherin germline mutations in familial gastric cancer.Nature. 392(6674):402-5, 1998